Aquacult. Sci. **72**(2), 157−160 (2024)

Short Paper

Effects of ultra-fine bubbles on the water quality in a closed recirculating system for tilapia *Oreochromis niloticus* and the microbiome in the rearing water

Yousuke TAOKA[1,*], Teruki KUROKI[2], Yasuhiro KODOI[2], Eisei SAKATA[2] and Chikashi KAMIMURA[2]

Abstract: We investigated the effects of ultra-fine bubbles (UFBs) on the water quality in a closed recirculating system for larval tilapia, *Oreochromis niloticus*, and the UFBs' effects on the water microbiome. The levels of inorganic nitrogen and phosphorus and chemical oxygen demand in the UFB group were maintained at lower level than those in the control group. A metagenomic analysis revealed that the ratio of Bacteroidetes increased, and that of Fusobacteria decreased in the rearing water in the UFB group compared to the control group. The study results thus indicate that the UFBs improved the water quality and changed the microbiome in the rearing water.

Key words: Ultra-fine bubble; Microbiome; Water quality; Closed recirculating system

The name "fine bubbles" has been defined by International Organization for Standardization (ISO), and fine bubbles are classified into micro bubbles and ultra-fine bubbles (UFBs). Micro bubbles have 1 to 100 μm diameters and become clouded. UFBs are bubbles of air as gas in a medium enclosed by an interface with a volume-equivalent diameter <1 μm; they are transparent and have unique physiological characteristics (ISO 2017). It has been reported that the radicals such as a hydroxyl radical (OH) can be generated by the production of UFBs (Liu et al. 2016). Water that contains UFBs is called 'UFB water', and several studies indicate the possibility of using UFB water for industrial purposes such as antibacterial activity against bacteria, decolorization of stains, acceleration of plant growth, seed germination, and waste water treatment (Attramadal et al. 2014; Puwanto et al. 2019).

Although fine bubble technology has advanced in Japan, information about the application of UFBs in the aquaculture field is scarce. Aquaculture methods have been expected to play a critical role in global food production, and compared to open-cage aquaculture systems, next-generation and sustainable approach is that of recirculating aquaculture system (RAS) (Ahmed and Turcchini 2021). In a RAS, the management of the water quality and the microbiota in the rearing water is very important (Attramandal et al. 2012). We conducted the present study to determine the effect of UFBs on the water quality and microbiome in an RAS for tilapia, which is the most frequently cultured freshwater fish worldwide.

We obtained larval tilapia, *Oreochromis niloticus*, with mean body weight of 7.6 g from a commercial company (Aquaponics, Kanagawa, Japan). The fish were transported to University of Miyazaki, Japan and conditioned in a closed recirculating system in an 80 *l* tank (working volume, 60 *l*) with a filter unit (Power box SV450X, Kotobuki kogei Co., Ltd., Nara, Japan) for 1 week prior to the start of rearing trial with or without UFBs. Two simultaneous rearing systems were set up, and 11 fish were reared in each tank. An Eatech SW (Eatech Co. Ltd., Kumamoto, Japan) was used for the generation of UFBs. This machine was operated at the level of air supply at 3.5 *l*/min for 6 h every day to generate UFBs. The mean size and numbers of the UFBs were measured by a NanoSight instrument (Malvern Panalytical, Malvern, England), and the result were 151.9 ± 92.2 nm and 3.06×10^8 particles ± 1.19 ± 10^7/m*l*, respectively. The peak of the size distribution was 90 nm. Aeration through an air-stone was carried out with an air-pump. In the control rearing system, only aeration was carried out without UFBs supplementation.

UFBs were supplied 24 h prior to rearing experiment. The fish were fed a commercial diet (Floating Diet, Marubeni Nisshin Feed Co., Tokyo, Japan) at 1% of the fishes' body weight for 36 days. Water samples were collected every 3 days for the measurement of water quality parameters. Rearing water (1 *l*) was filtrated with a 0.22-μm MF-Millipore filter (Merck, Darmstadt, Germany) to collect bacterial cells on the filter.

Received 26 December 2022; Accepted 29 March 2024.
[1] Department of Marine Biology and Environmental Sciences, Faculty of Agriculture, University of Miyazaki, Gakuen-kibanadai-Nishi 1-1, Miyazaki 889-2192, Japan.
[2] Eatech Co. Ltd., Matsuzaki 159-1, Yatsushiro, Kumamoto 866-0884, Japan.
[*] Corresponding author: Tel, (+81) 985-58-7232; E-mail, yousuketao@cc.miyazaki-u.ac.jp (Y. Taoka).

In water quality analyses, ammonia-nitrogen (NH_4-N), nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N) and phosphate-phosphorus (PO_4-P) were analyzed by the method of Strickland and Parsons (1972). Total inorganic nitrogen (TIN) was calculated as the sum of NH_4-N, NO_2-N, and NO_3-N. The chemical oxygen demand (COD) was analyzed by a standard method using alkaline potassium permanganate.

After the rearing water was filtrated, the filter was cut into small pieces and then placed in a 2.0-m*l* sterilized Eppendorf tube. The bacterial genome DNA was extracted by the cetyl trimethylammonium bromide (CTAB) method with slight modification (Coyne et al. 2005; Mirimin and Roodt Wilding 2015). The polymerase chain reaction analysis of the V3/V4 region in 16S rRNA gene, sequencing, and metagenomics analysis of bacterial flora in the rearing water were outsourced to Bioengineering Lab. Co., Kanagawa, Japan. In brief, the concentration of the obtained libraries were measured with a Synergy H1 (Agilent Technologies, Inc., USA) and QuantiFluor dsDNA System (Promega Corporation, USA). The quality of the libraries was checked with a Fragment Analyzer using dsDNA 915 Reagent Kit (Agilent Technologies, Inc., USA), and sequencing was carried out with a MiSeq system and MiSeq Reagent Kit v 3 (Illumina, San Diego, CA, USA). The data processing and assignment was carried out based on Qiime 2 (ver. 2023.7).

The average fish body weight at start of rearing trial (day 0) was 7.8 ± 1.7 g. On day 36, the average fish body weight in the control group and the UFB group were 15.0 ± 4.2 and 16.3 ± 3.5 g, respectively. The average fish body length in the control and UFB groups were 101 ± 9 and 102 ± 8 mm, respectively. The results of water quality analyses are depicted in Fig. 1. The concentration of NH_4-N in the control group was greatly increased on day 15 and rose by up to 1.8 mg/*l* from 0.2 mg/*l* on day 21. The concentration of NH_4-N in the UFB group was stable at <0.3 mg/*l*

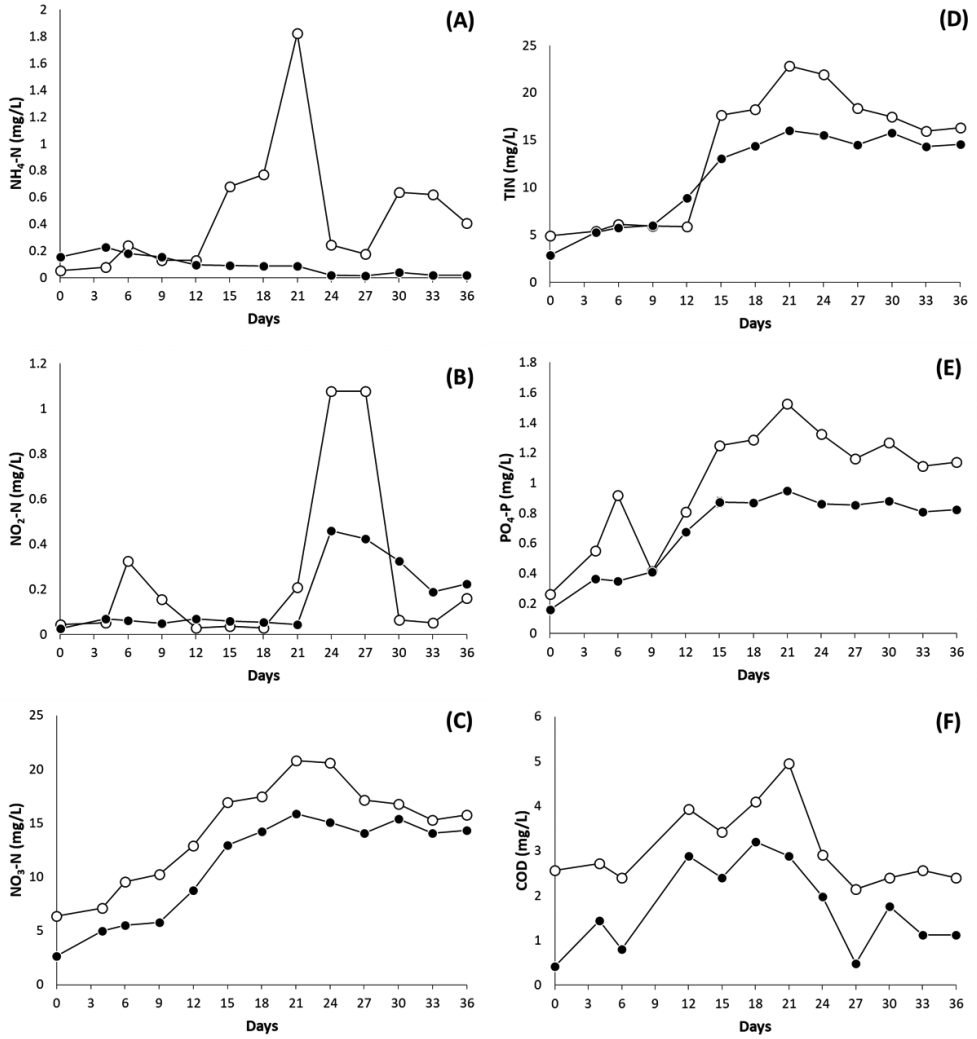

Fig. 1. Change of water quality parameters in the rearing water for 35 days of culturing tilapia with or without ultra-fine bubbles (UFBs). (A) ammonia nitrogen, (B) nitrite nitrogen, (C) nitrate nitrogen, (D) total inorganic nitrogen, (E) phosphate phosphorus, (F) Chemical oxygen demand. ○, control group; ●, UFB group.

over 36 days of rearing (Fig. 1A). The concentration of NO_2-N in the control group increased markedly to 1.1 mg/l by day 24 from 0.02 mg/l on day 18. The concentration of NO_2-N in the UFB group had also increased to 0.48 mg/l on day 24, but obviously it was lower than the concentration in the control group (Fig. 1B). The concentration of NO_3-N in the UFB group were lower than that in the control group throughout 36 days of rearing (Fig. 1C). TIN in the UFB group was lower than that of the control group on days 15-36 (Fig. 1D). The concentration of phosphate measured as phosphorus (PO_4-P) in the UFB group was clearly lower than that in the control group throughout the 36 days of rearing except for day 9 (Fig. 1E). The COD in the UFB group was clearly lower than that in the control group throughout the 36 days (Fig. 1F). After 36 days, brownish adhesions attached to the inside wall of the rearing tank were observed in the control group compared to in the UFB group (Fig. 2).

Fig. 3 shows the rearing water's composition of microbiota at the phylum level. High-throughput sequencing yielded 30,099 reads in the control group and 30,126 leads in the UFB group. In both groups, Proteobacteria, Bacteroidetes and Fusobacteria were observed as major predominant bacterial groups, and the sum of the relative ratio was 88% in the control group and 95% in the UFB group. In the UFB group, the ratio of Bacteroidetes was increased and that of Fusobacteria decreased compared to those in the control group (Fig. 3)

The water quality analysis revealed that the concentrations of inorganic nitrogen and phosphorus, and the COD were kept at lower level compared to those in the control group by the supply of UFBs. Akter et al. (2022) reported that organic compounds can be removed by the radical oxidation based on OH. The metagenomic analysis also revealed that the ratios of Bacteroidetes and Fusobacteria were increased and decreased, respectively by UFB supplementation compared to the control group's value (Fig. 3). Chen et al. (2019) reported that Bacteroidetes were one of the dominant bacterial groups in a RAS for shrimp, and Bacteroidetes have been known as a wide range of complex carbohydrates such as polysaccharide-degrading bacteria (McKee et al. 2021). It has been reported that some species of Fusobacteria have virulence against salmonid fish (Nematollahi et al. 2003).

In summary, the results of our present experiment showed that UFB supplementation in a closed recirculating system improved the water quality and modified the microbiome in the rearing water.

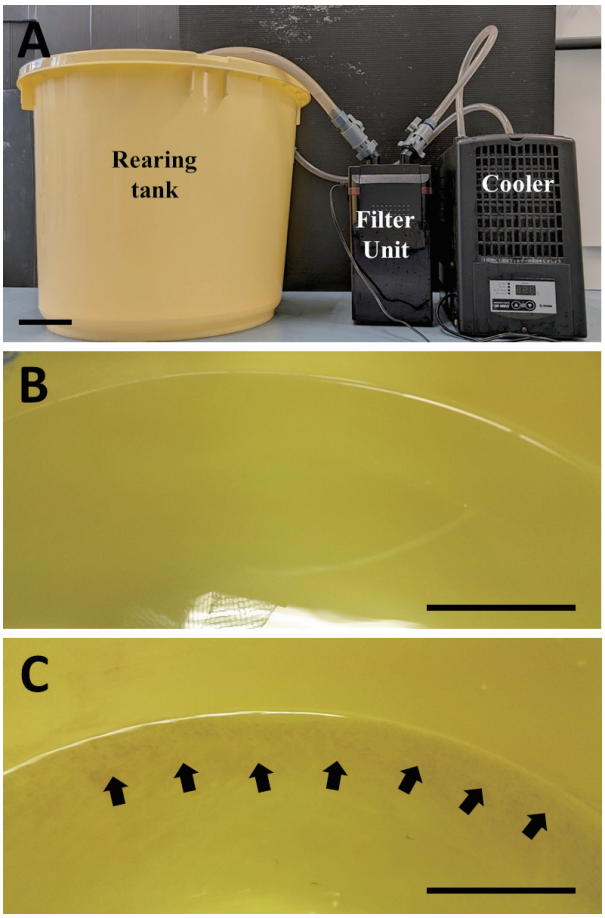

Fig. 2. Pictures of closed recirculating system used for rearing trial (A) and the inside wall of the rearing tanks on day 36 after start of rearing experiment with (B) or without ultra-fine bubble (C). Arrows show brownish adhesions attached to the inside wall of the rearing tank in the control group. Scales; 100 mm.

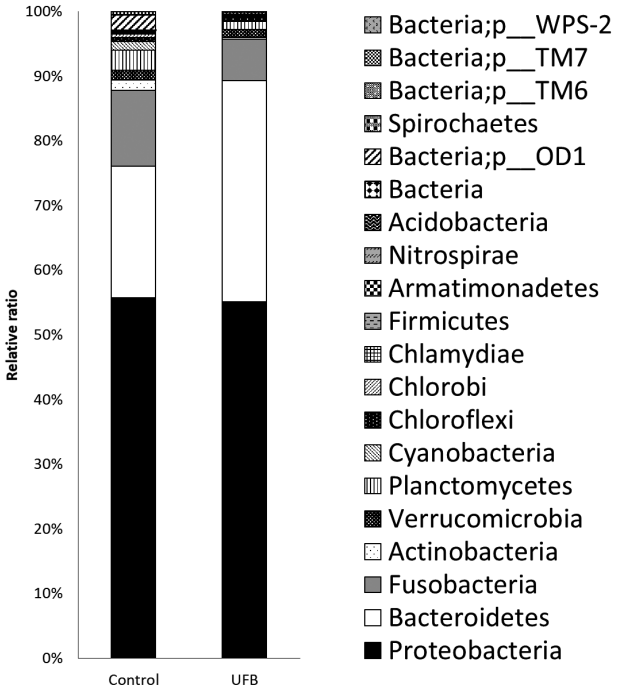

Fig. 3. Composition of microbiota in the rearing water at phylum level. Values show relative ratio (percentage composition) of each bacterial group. UFB: ultra-fine bubble.

References

Ahmed, N. and G. M. Turcchini (2021) Recirculating systems (RAS): environmental solution and climate change adaptation. *J. Clean. Prod*., **297**, 126604.

Akter, J., J. Y. Lee, H. J. Ha, I. G. Yi, D. H. Hong, C. M. Park, M. Y. Lee and I. Kim (2022) Degradation of organics and change concentration in per-fluorinated compounds (PFCs) during ozonation and UV/H_2O_2 advanced treatment of tertiary-treated sewage. *Sustainability*, **14**, 5597.

Attramandal, K. J. K., I. Salvesen, R. Xue, G. Øie, T. R. Størseth, Vadstein, O. and Y. Olsen (2012) Recirculation as a possible microbial control strategy in the production of marine larvae. *Aquac. Eng.*, **46**, 27-39.

Attramadal, K. J., T. M. H. Truong, I. Bakke, J. Skjermo, Y. Olsen and O. Vadstein (2014) RAS and microbial maturation as tools for K-selection of microbial communities improve survival in cod larvae. *Aquaculture*, **432**, 483-490.

Chen Z., Z. Chang, L. Zhang, Y. Jiang, H. Ge, X. Song, S. Chen, F. Zhao and J. Li (2019) Effects of water recirculation rate on the microbial community and water quality in relation to the growth and survival of white shrimp (*Litopenaeus vannamei*). *BMC Microbiol*., **19**, 192.

Coyne K. J., S. M. Handy, F. Demir, E. B. Whereat, D. A. Hutchins, K. J. Portune, M. A. Doblin and S. C. Cary (2005) Improved quantitative real-time PCR assays for enumeration of harmful algal species in field samples using an exogenous DNA reference standard. *Limnol Oceanogr-METH*, **3**, 381-391.

ISO (2017) Fine bubble technology − General principles for usage and measurement of fine bubbles part 1: Terminology. 20480-1, Geneva, Switzerland, https://www.iso.org/obp/ui/#iso:std:iso:20480:-1:ed-1:v1, 2 December 2022.

Liu, S., S. Oshita, S. Kawabata, Y. Makino and T. Yoshimoto (2016) Identification of ROS produced by nanobubbles and their positive and negative effects on vegetable seed germination. *Langmuir*, **32**, 11295-11302.

McKee, L. S., S. L. La Rosa, B. Westereng, V. G. Eijsink, P. B. Pope and J. Larsbrink (2021) Polysaccharide degradation by the Bacteroidetes: mechanisms and nomenclature. *Environ. Microbiol. Rep.*, **13**, 559-581.

Mirimin, L. and R. Roodt Wilding (2015) Testing and validating a modified CTAB DNA extraction method to enable molecular parentage analysis of fertilized eggs and larvae of an emerging South African aquaculture species, the dusky kob *Argyrosomus japonicus*. *J. Fish. Biol.*, **86**, 1218-1223.

Nematollahi, A., A. Decostere, F. Pasmans and F. Haesebrouck (2003) *Flavobacterium psychrophilum* infections in salmonid fish. *J. Fish Dis.*, **26**, 563-574.

Puwanto, Y. A., N. N. Maulana, Sobir, Sulassih and N. Naibaho (2019) Effect of ultrafine bubbles water on seed germination. *IOP Conf. Ser.: Earth Environ. Sci.*, **355**, 012073.

Strickland, J. D. H. and T. R. Parsons (1972) A practical handbook of seawater analysis. *Bull. Fish. Res. Bd. Can.*, **167**, 1-311.